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# N-METHYL-D,L-ASPARTATE MODULATION OF PITUITARY HORMONE SECRETION IN THE PIG: ROLE OF OPIOID PEPTIDES

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## ABSTRACT

Sixteen ovariectomized (OVX) mature gilts, averaging 139.6 ± 3.1 kg body weight (BW) were assigned randomly to receive either progesterone (P, 0.85 mg/kg BW, n=8) or corn oil vehicle (OIL, n=8) injections im twice daily for 10 d. On the day of experiment, all gilts received either the EAA agonist, N-methyl-d,l-aspartate (NMA; 10 mg/kg BW, iv) alone or NMA plus the EOP antagonist, naloxone (NAL, 1 mg/kg BW, iv), resulting in the following groups of 4 gilts each: OIL-NMA, OIL-NMA-NAL, P-NMA and P-NMA-NAL. Blood samples were collected via jugular cannula every 15 min for 6 hr. All pigs received NMA 5 min following pretreatment with either 0.9% saline or NAL 2 hr after blood collection began and a GnRH challenge 3 hr after NMA. Administration of NMA suppressed ($P<0.03$) LH secretion in OIL-NMA gilts and treatment with NAL failed to reverse the suppressive effect of NMA on LH secretion in OIL-NMA-NAL gilts. Similar to OIL-NMA gilts, NMA decreased ($P<0.03$) mean serum LH concentrations in P-NMA gilts. However, in P-NMA-NAL gilts, serum LH concentrations were not changed following treatment. All gilts responded to GnRH with increased ($P<0.01$) LH secretion. Additionally, administration of NMA increased ($P<0.01$) growth hormone (GH) and prolactin (PRL) secretion in both OIL-NMA and P-NMA gilts, but this increase in GH and PRL secretion was attenuated ($P<0.01$) by pretreatment with NAL in OIL-NMA-NAL and P-NMA-NAL gilts. Serum cortisol concentrations increased ($P<0.01$) in all gilts and the magnitude of the cortisol response was not different among groups. In summary, results of the present study confirmed previous findings that NMA suppresses LH secretion in both oil- and P-treated OVX gilts, but we failed to provide definitive evidence that EOP are involved in the NMA-induced suppression of LH secretion. However, NMA may, in part, activate the EOP system which in turn increased GH and PRL secretion in the gilt.

## INTRODUCTION

An abundance of information suggests a general stimulatory role of excitatory amino acids (EAA) on release of neuropeptides from the central nervous system, which in turn influence pituitary functions. In many species, including the sheep (1,2), rat (3,4,5) and primate (6,7), administration of an EAA analogue, N-methyl-d,l-aspartate (NMA), stimulated LH secretion, possibly through activation of EAA receptors on GnRH neurons within the hypothalamus (8,9). Similarly, NMA facilitated growth hormone (GH) and prolactin (PRL) secretion in several species (10-13). In contrast, an unexpected inhibition of LH secretion by NMA was observed in pigs (12) and monkeys (7,14) and was associated with a concomitant increase in cortisol secretion. In the monkey, the involvement of corticotropin-releasing factor (CRF) and endogenous opioid peptides (EOP) in mediating this effect has been suggested,

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because pretreatment with CRF antiserum or the EOP antagonist naloxone (NAL), blunted the NMA-induced LH decrease (14). Previous studies in the pig have demonstrated that EOP stimulate GH and PRL secretion, but suppress LH secretion (15,16). Therefore, the objective of the present study was to determine whether the EOP system mediates the effect of NMA on pituitary hormone secretion in the pig.

## MATERIALS AND METHODS

Sixteen mature gilts, averaging 139.6 ± 3.1 kg body weight (BW) and exhibiting one or more estrous cycles of 18-22 d were bilaterally ovariectomized (OVX). They were assigned randomly to receive either progesterone (P, 0.85 mg/kg BW, n=8) or corn oil vehicle (OIL, n=8) injections im twice daily for 10 d prior to the experiment. The dose of P was employed in order to simulate luteal phase concentrations of P (17). On the last day of P treatment, all gilts received either NMA[1] (10 mg/kg BW, iv) alone or NMA in combination with NAL[2] (1 mg/kg BW, iv) in a 2X2 factorial arrangement of treatments with the main effects of steroids (P *vs* OIL) and treatments (NMA *vs* NMA+NAL), resulting in the following groups of 4 gilts each: OIL-NMA, OIL-NMA-NAL, P-NMA and P-NMA-NAL. Doses of NMA and NAL used in present study were determined previously (12,17).

On the day prior to NMA and NAL administration, an indwelling catheter was placed into a jugular vein of each pig with an 11-gauge thin-walled hypodermic needle without the use of an anaesthetic. Cannulae were secured to the outstide of the neck with 7.6 cm-wide elastic bandage wrap (18,19). Blood samples were collected every 15 min for 6 hr. All pigs received NMA approximately 5 min following pretreatment with either 0.9% saline or NAL 2 hr after blood collection began and a GnRH[3] (0.2 μg/kg BW,iv) challenge 3 hr after NMA. Blood samples were allowed to clot overnight at 4°C and serum was harvested after centrifugation and stored at -20°C.

**Hormone assays:** Serum concentrations of LH (20), PRL (19), and GH (21) were quantified by radioimmunoassay (RIA). Serum cortisol concentrations were determined by RIA (12) for samples collected from all gilts immediately prior to and during the first hr after the NMA injection. Assay sensitivities for LH, PRL, GH and cortisol were 0.15 ng/ml, 1 ng/ml, 0.4 ng/ml and 1 ng/ml, respectively. Intraassay and interassay coefficients of variation were 4.8% and 9.0% for LH, 16.3% and 15.2% for PRL, 3.2% and 13.6% for GH and 1.4% and 4.4% for cortisol, respectively. Serum P concentrations were quantified by RIA (22) for the sample prior to NMA injection. The sensitivity of the P assay was 0.5 ng/ml and intraassay and interassay coefficients of variation were 9.3% and 15.0%, respectively.

**Statistical analysis:** To determine the effect of NMA and NMA+NAL treatments on LH, PRL and GH secretion, sampling time was divided into five periods. Period one represents the mean of samples collected prior to treatment. The remainder of the sampling time was divided into four 1-hr periods. Data were then subjected to the general linear model split plot-in-time analysis of variance procedures of the Statistical Analysis System (SAS; 23). Data were analysed with steroid, treatment, pig, and period as discrete (class) variables. Steroid, treatment and steroid X treatment interaction were tested using pig within treatment X steriod as the error term. Period, treatment X period, steroid X period and steroid X treatment X period interactions were tested using period X pig within treatment X steroid as the error term. Differences between treatments within steroid group or within a treatment between periods were determined by least squares contrasts. Serum cortisol data were analyzed using the same statistical model with period replaced by sample as the source of variance.

## RESULTS

Initial behavior following NMA treatment varied between gilts and included abdominal contractions and emesis (n=4), immobile while standing and increased salivation (n=6). These reactions lasted for approximately 10 min. Serum P concentrations averaged 34.5 ± 5.1 ng/

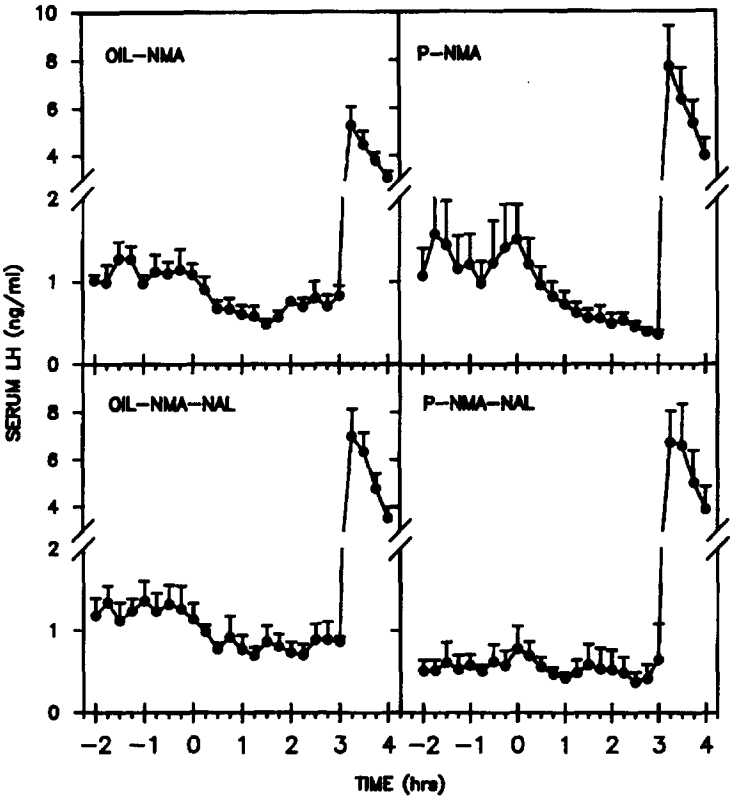


Figure 1. Mean serum luteinizing hormone (LH) concentrations before and after N-methyl-d,l-aspartate (NMA) or naloxone (NAL) pretreatment followed by NMA (treatment = Time 0) and GnRH (hr 3) for gilts treated with oil vehicle (OIL-NMA or OIL-NMA-NAL) or progesterone (P-NMA or P-NMA-NAL).

ml and 26.7 ± 1.5 ng/ml for P-NMA and P-NMA-NAL gilts, respectively, and were nondectable in OIL-NMA and OIL-NMA-NAL gilts. A treatment X steroid X period interaction was detected ($P<0.05$) for LH (Figure 1). Serum LH concentrations decreased ($P<0.03$) after NMA in OIL-NMA gilts and remained suppressed for the next 3 hr compared to pretreatment concentrations (Figure 1). Naloxone treatment failed to reverse the suppressive effect of NMA on LH secretions in OIL-NMA-NAL gilts (Figure 1). Serum LH concentrations decreased ($P<0.03$) after treatment in the P-NMA gilts (Figure 1). However, LH secretion was unchanged by treatment in the P-NMA-NAL gilts (Figure 1). All gilts responded to the GnRH challenge with a marked increase ($P<0.01$) in serum LH concentrations.

A treatment X period interaction was detected ($P<0.0001$) for PRL. Serum PRL concentrations in P-NMA and P-NMA-NAL gilts were lower ($P<0.01$) than OIL-NMA and OIL-NMA-NAL gilts during the pretreatment period (Figure 2). NMA elevated ($P<0.01$) PRL concentrations in OIL-NMA and P-NMA gilts during the first hr after treatment when compared to pretreatment levels. However, NAL suppressed ($P<0.01$) the PRL response to NMA in P-NMA-NAL and OIL-NMA-NAL animals when compared to P-NMA and OIL-NMA gilts, respectively (Figure 2). Furthermore, attenuation of NMA-induced PRL secretion by NAL was greater in ($P<0.01$) in P-NMA-NAL gilts than in OIL-NMA-NAL gilts.

Treatment X period ($P<0.002$) and steroid X period ($P<0.05$) interactions were detected for GH. Serum GH concentrations were similar among all treatment groups during the pretreatment period (Figure 3). NMA increased ($P<0.01$) serum GH concentrations in OIL-NMA and P-NMA animals. Serum GH concentrations were higher ($P<0.01$) during the first hr after NMA treatment in P-NMA and P-NMA-NAL gilts compared to OIL-NMA and OIL-

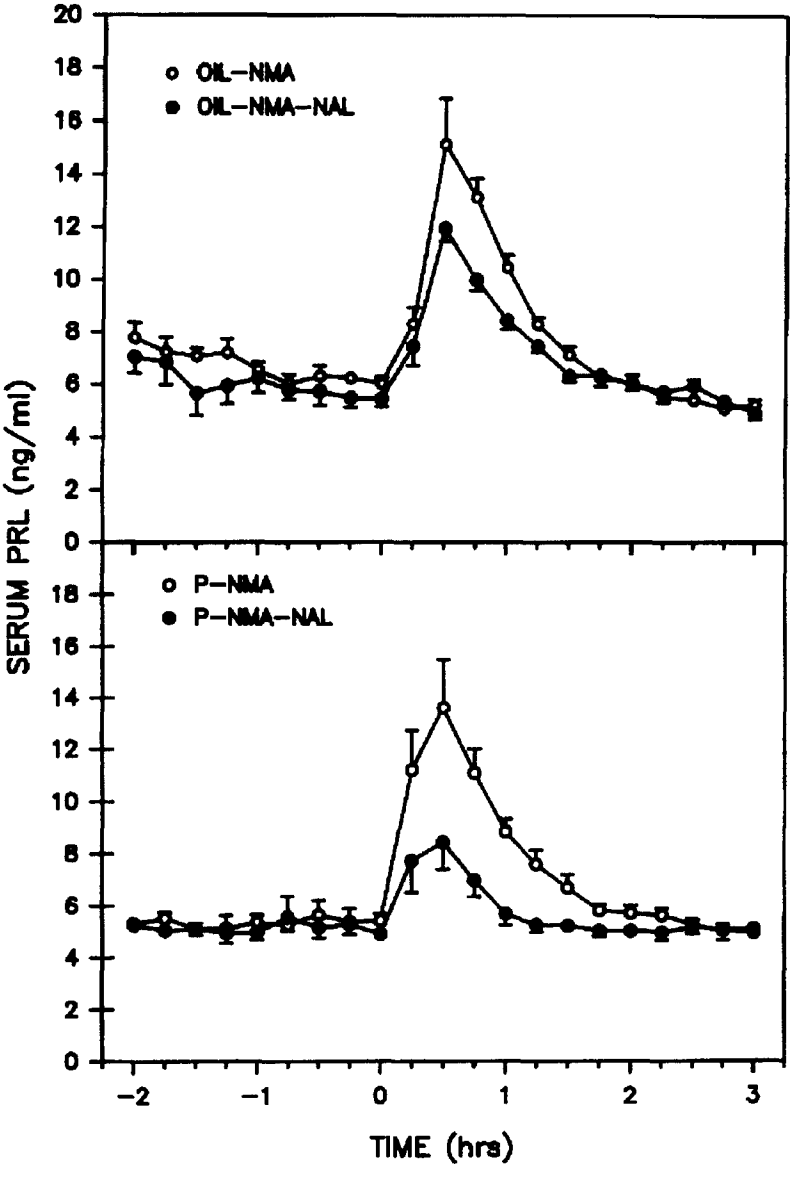


Figure 2. Mean serum prolactin (PRL) concentrations before and after N- methyl-d,l-aspartate (NMA) or naloxone (NAL) pretreatment followed by NMA (treatment = Time 0) for gilts treated with oil vehicle (OIL-NMA or OIL-NMA-NAL) or progesterone (P-NMA or P-NMA-NAL).

NMA-NAL gilts, respectively. However, NAL pretreatment attenuated ($P<0.01$) the GH response to NMA in OIL-NMA-NAL and P-NMA-NAL groups when compared to OIL-NMA and P-NMA gilts, respectively (Figure 3).

Serum cortisol concentrations increased ($P<0.01$) by 15 min after NMA in all groups and remained elevated. Naloxone treatment prior to NMA failed to alter the secretory profiles of cortisol when compared to NMA alone (Figure 4).

## DISCUSSION

As in our previous study (12), results from the present experiment demonstrated NMA inhibited LH secretion in gilts, a finding which is in contrast to the excitatory role of NMA on LH secretion in other species (1-6). Moreover, Sesti and Britt (24) reported that NMA increased LH secretion in estrogen-treated pigs, but not in pigs pretreated with antiserum

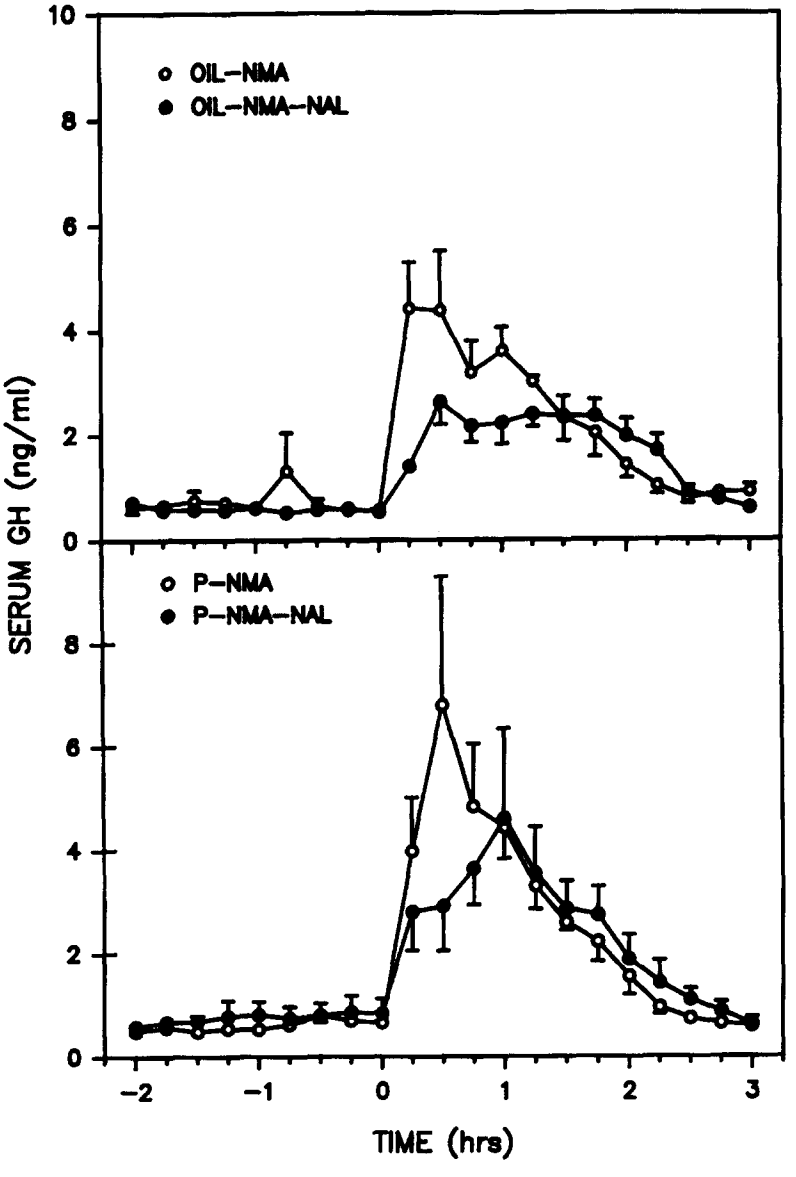


Figure 3. Mean serum growth hormone (GH) concentrations before and after N-methyl-d,l-aspartate (NMA) or naloxone (NAL) pretreatment followed by NMA (treatment = Time 0) for gilts treated with oil vehicle (OIL-NMA or OIL-NMA-NAL) or progesterone (P-NMA or P-NMA-NAL).

to GnRH. It is possible that estrogen is a necessary antecedent for the excitatory action of NMA on LH secretion in the pig. However, in our previous study (12) which utilized a similar paradigm as Sesti and Britt (24), NMA failed to alter LH concentrations in the estrogen treated gilt. The only explanation for this paradox is the difference in time after OVX when the two studies were conducted. Moreover, the inhibitory action of NMA on LH secretion was previously observed only in progesterone and oil-treated anaimals (12). Therefore, in the present study only these treatment groups were utilized.

It has been previously suggested that NMA may activate the EOP system, which causes reduction of LH secretion in the pig (12) and monkey (7). This concept is supported by findings of Reyes et al. (14) and Bach et al. (25). In the OVX monkey, NMA suppression of LH secretion was attenuated by the EOP antagonist, naloxone (14). Furthermore, beta-endorphin concentrations increased in cerebral spinal fluid in rats microinjected with NMA

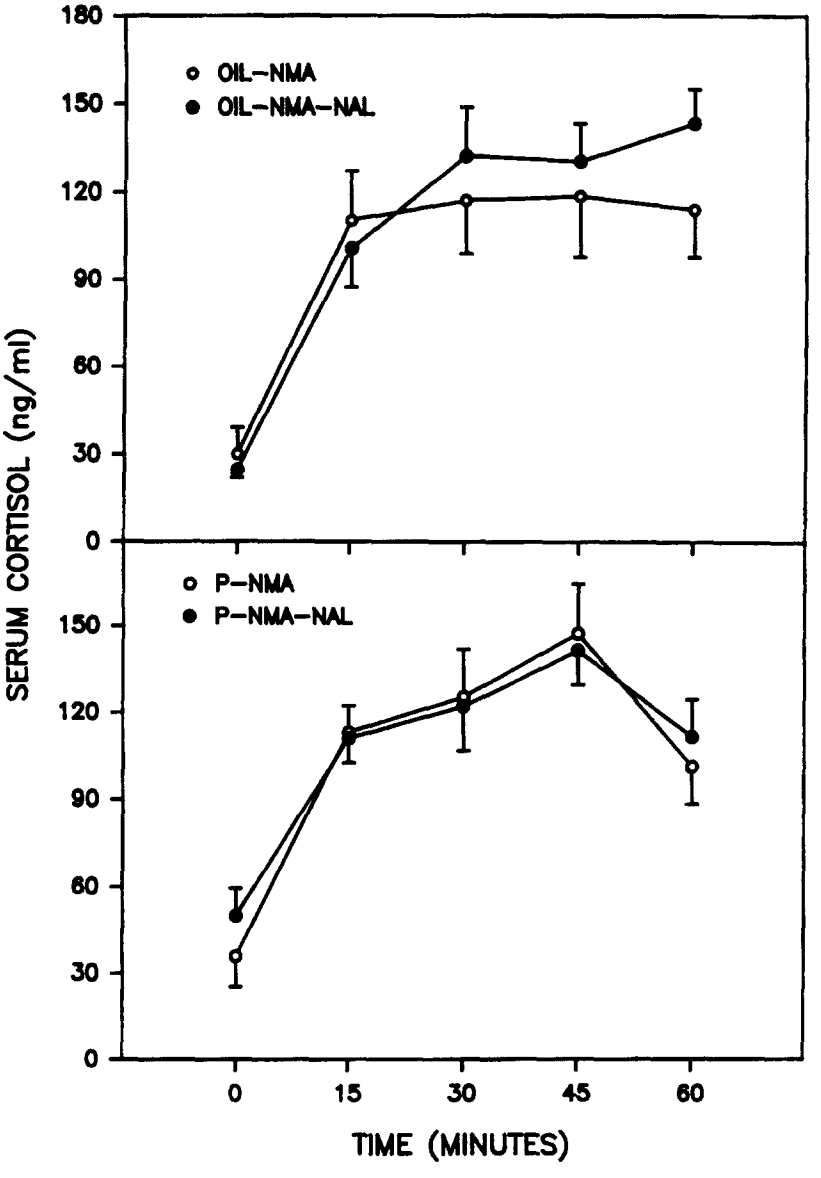


Figure 4. Serum cortisol concentrations (mean ± SE) during the first hr after N-methyl-d,l-aspartate (NMA) or naloxone (NAL) pretreatment followed by NMA (time 0) for gilts treated with oil vehicle (OIL-NMA or OIL-NMA-NAL; panel A) or progesterone (P-NMA or P-NMA-NAL; panel B). All sample times differ from time 0 ($P<0.01$).

into the arcuate nucleus (25). In contrast, in the present study, NAL failed to reverse the NMA-induced decrease in LH secretion in OIL-NMA-NAL gilts. These conflicting results could be due to different methods of NAL administration. In the study of Reyes et al. (14), NAL was infused iv prior to and during NMA treatment, but the negative effect of NMA on LH secretion was not completely blocked, whereas in the present study NAL was administered as a bolus injection just prior to NMA treatment. A higher dose of NAL or a longer time of exposure to NAL may be required to antagonize NMA inhibition of LH secretion. In addition, since in the P-NMA-NAL gilts serum LH concentrations were suppressed during the pretreatment period, it is difficult to conclude whether NMA or NAL had any effect on LH secretion. Therefore, EOP modulation of the NMA-induced suppression of LH secretion can not be dismissed.

Alternatively, NMA may modulate LH secretion through activation of the hypothalamic-pituitary-adrenal axis. Plasma adrenocorticotropin (ACTH) concentration increased after NMA in the rat (26,27). In addition, Pearce et al. (28) demonstrated in the pig, that within 30 to 45 min after acute elevation of plasma cortisol concentrations the LH response to GnRH was reduced. Moreover, the cortisol induced suppression of LH secretion in the pig (29) is believed to occur at the central nervous system. In the present study, an abrupt increase in serum cortisol concentrations was concomitant with decreased serum LH concentration after NMA. Together with previous findings, these data suggest that elevated cortisol concentrations after NMA may mediate subsequent reduction in LH secretion observed after NMA treatment.

Much evidence has indicated an important role for EAA and EOP in modulation of GH secretion (10,11,12,16). It is believed that, in general, GH secretion in response to EOP is mediated by growth hormone-releasing factor (GRF; 30). Barb et al. (12) suggested that NMA may stimulate GH secretion via activating the EOP system. This hypothesis was confirmed by results of the present study in which NAL pretreatment suppressed the NMA-induced increase in GH secretion. In contrast, Estienne et al. (31) failed to demonstrate EOP involvement in the NMA-induced release of GH in the ewe. This apparent discrepancy between the two studies may in part be due to a species difference in the role of EOP and EAA in modulating GH secretion. Furthermore, in the present study, a single injection of NAL did not completely block the GH response to NMA, implying that a higher dose or longer time of exposure to NAL may be required and/or possible involvement of other neural systems in modulating GH secretion in the pig. Future studies are required to determine the nature of these putative neurotransmitters.

Finally, results of the present study indicate a role for P in modulating the GH response to NMA and NAL, as reflected by higher GH concentrations during the first hr after NMA treatment in P-NMA and P-NMA-NAL gilts compared to OIL-NMA and OIL-NMA-NAL gilts. Moreover, the greater GH response to NMA injection in P-NMA and P-NMA-NAL gilts could be due to enhanced sensitivity of the GRF/GH secretory system to NMA in P-treated gilts.

In concert with other reports (13,32) and similar to a previous report by investigators from this laboratory (12), NMA increased serum PRL concentrations in all gilts. It is possible that NMA may stimulate PRL secretion via EOP inhibition of dopaminergic neuronal activity. In the present study, PRL response to NMA was blunted by NAL pretreatment in both OIL-NMA-NAL and P-NMA-NAL gilts, indicating that a stimulatory EOP neuronal pathway may be involved in NMA-induced PRL secretion in the gilt. A similar role for EOP in modulating PRL secretion has been previously reported for the lactating sow (15).

In addition, a P-dependent EOP system which inhibits PRL secretion has been previously demonstrated in the pig (15,16). In the present study, serum PRL concentrations during the pretreatment period were lower in P-treated gilts (P-NMA and P-NMA-NAL) than in oil-treated gilts (OIL-NMA and OIL-NMA-NAL). Furthermore, attenuation of NMA-induced PRL secretion by NAL was more pronounced in P-NMA-NAL gilts than in OIL-NMA-NAL gilts. As mentioned above, P reinitiated the EOP inhibition of PRL secretion in the pig. Therefore, it is possible that the difference in basal PRL concentrations and PRL response to NAL in OIL-NMA-NAL and P-NMA-NAL groups may in part be due to changes in sensitivity of intermediate neurons, which release dopamine or thyrotropin-releasing hormone, in response to EOP stimulation under the influence of progesterone.

In summary, we have demonstrated that a single injection of NAL markedly attenuated the GH and PRL responses to NMA, but failed to reverse the NMA-induced decrease in LH secretion. Moreover, we have demonstrated a differential response of GH and PRL to NMA and NMA-NAL treatments between the oil- and P-treated gilts. These data indicate that interactions of EOP and EAA may have physiological importance in modulation of pituitary hormone secretion.

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